

WHAT IS CLAIMED IS:

1. A method for identifying inhibitors of neuronal degeneration comprising (a) cotransfected eukaryotic host cells expressing a presenilin protein (PS), with a polynucleotide encoding a Par-4 polypeptide, and an NF- κ B dependent reporter construct, (b) exposing the cotransfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.
- 5 2. The method of claim 1 wherein said eukaryotic host cells are mammalian cells endogenously expressing PS.
- 10 3. The method of claim 1 wherein said eukaryotic host cells are mammalian cells transfected with nucleic acid encoding PS.
4. The method of claim 3 wherein said PS is PS1.
5. The method of claim 4 wherein said PS1 is human.
6. The method of claim 3 wherein said PS is FAD PS.
7. The method of claim 6 wherein said FAD PS is FAD PS1.
- 15 8. The method of claim 7 wherein said FAD PS1 is human.
9. The method of claim 1 wherein said eukaryotic host cells are neuronal cells.
10. The method of claim 9 wherein said neuronal cells are cerebellar granule cells.
11. The method of claim 9 wherein said neuronal cells are organotypic brain cells obtained from transgenic mice genetically engineered to express human PS1.
- 20 12. The method of claim 11 wherein said human PS1 is FAD PS1.
13. The method of claim 1 wherein said NF- κ B dependent reporter construct comprises a luciferase reporter gene.
14. The method of claim 13 wherein said NF- κ B dependent reporter construct comprises NF- κ B-binding consensus sites linked to a luciferase reporter gene.
- 25 15. The method of claim 1 wherein the ability of said candidate molecule to induce NF- κ B activation is monitored in comparison with a known inducer of NF- κ B activation.
16. The method of claim 15 wherein said known inducer of NF- κ B activation is TNF- α .

17. The method of claim 1 wherein the cotransfected cells are exposed to a plurality of candidate molecules.

18. The method of claim 1 further comprising the step of administering an identified inhibitor to a patient suffering from or at risk of acquiring a neurodegenerative disease.

19. The method of claim 18 wherein said neurodegenerative disease is Alzheimer's disease.

20. A method for identifying inhibitors of neuronal degeneration, comprising (a) transfecting eukaryotic host cells endogenously expressing Par-4 and a presenilin (PS) protein with nucleic acid encoding an NF- κ B dependent reporter construct, (b) exposing the transfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.

21. The method of claim 20 wherein said eukaryotic host cells are HeLa cells.

22. The method of claim 20 wherein the transfected cells are exposed to a plurality of candidate molecules.

23. A method for identifying inhibitors of Par-4 expression or activity comprising (a) transfecting eukaryotic host cells endogenously expressing Par-4 and a presenilin (PS) protein with nucleic acid encoding an NF- κ B dependent reporter construct, (b) exposing the transfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.

24. A method for identifying inhibitors of Par-4 expression or activity comprising (a) transfecting mammalian cells with nucleic acid comprising a Par-4 promoter region fused to a reporter gene, (b) exposing said cells to a pro-apoptotic agent followed by exposure to a candidate molecule, and (c) monitoring the ability of said candidate molecule to inhibit the activity of said reporter gene.

25. The method of claim 24 wherein said Par-4 gene is of human origin.

26. The method of claim 24 wherein said reporter gene is a luciferase gene.

27. The method of claim 24 wherein said cell is a cell line endogenously expressing Par-4.

28. The method of claim 27 wherein said cell line is a HeLa cell line.

29. The method of claim 24 wherein said cell is exposed to a plurality of candidate molecules.

30. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring the NF- κ B DNA binding activity in the cell extract.

5 31. The method of claim 30 wherein NF- κ B DNA binding activity is monitored by electrophoretic mobility shift assay.

10 32. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring ξ PKC in the cell extract.

33. The method of claim 32 wherein said ξ PKC is monitored by an enzymatic assay.

15 34. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring the level of I κ B kinase (IKK) phosphorylation.

35. The method of claim 34 wherein the level of I κ B kinase (IKK) phosphorylation is measured by metabolic labeling and immunoprecipitation.

36. The method of claim 35 wherein immunoprecipitation of the cell extract is performed with IKK specific antibodies.

20 37. A method for identifying inhibitors of neuronal degeneration comprising (a) transfecting a mammalian cell with nucleic acid comprising a Par-4 promoter region fused to a reporter gene, (b) exposing said cell to a pro-apoptotic agent followed by exposure to a candidate molecule, and (c) monitoring the ability of said molecule to inhibit the activity of said reporter gene.

38. An isolated nucleic acid molecule comprising a Par-4 promoter region.

39. The nucleic acid molecule of claim 38 wherein said Par-4 is of human origin.

40. An expression vector comprising a Par-4 promoter region.

41. The expression vector of claim 40 further comprising nucleic acid encoding a heterologous polypeptide under the control of said Par-4 promoter region.

42. The expression vector of claim 41 further comprising nucleic acid encoding a reporter gene under the control of said Par-4 promoter region.

43. The expression vector of claim 42 wherein said reporter gene is a luciferase gene.

5 44. A recombinant host cell transformed with an expression vector comprising nucleic acid encoding a heterologous polypeptide under the control of a Par-4 promoter region.

45. A method for producing a heterologous polypeptide comprising transforming a host cell with nucleic acid comprising the coding sequence of said polypeptide under 10 control of a Par-4 promoter region and culturing the transformed host cell.

46. A method for identifying inhibitors of neuronal degeneration comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, (b) exposing said cell to a pro-apoptotic agent, and (c) monitoring ξ PKC in the cell extract.

47. The method of claim 46 wherein said ξ PKC is monitored by an enzymatic 15 assay.

48. A method of inhibiting Par-4 activity in eukaryotic cells comprising introducing into said cells a nucleic acid comprising a Par-4 promoter region.

49. A method of preventing neuronal degeneration in a mammal comprising introducing into said mammal a nucleic acid comprising a Par-4 promoter region.

20 50. A method of preventing neuronal degeneration in a mammal comprising introducing into said mammal an antisense nucleic acid comprising a sequence complementary to a Par-4 promoter region.

51. Inhibitors of neuronal degeneration identified by the method of any of claims 1, 20, 30, 32, 34, 37 and 46.

25 52. A process for obtaining a compound for the treatment of neuronal degeneration in a mammal, said process comprising:

screening a plurality of compounds for their ability to inhibit Par-4 activity; and preparing a pharmaceutical composition comprising one or more of said compounds identified in the screening and a suitable pharmaceutically acceptable carrier.